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Electroporation Studied by Molecular Dynamics Simulations

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Membrane electroporation is the method to directly transfer bioactive substances such as drugs and genes into living cells, as well as preceding electrofusion. Although much information on the microscopic mechanism has been obtained both from experiment and simulation, the existence and nature of possible intermediates is still unclear. Here, we investigated the equilibrium effect of external electric fields on the membrane structure, the effect of membrane-embedded proteins on the stability of membranes, and the kinetics of the electropore formation process, applying molecular dynamics simulations.

The results show a quadratic change of the membrane capacitance with the applied voltage, and a significant stabilization of membranes by proteins. For electropores, an average pore radius of 0.47 ± 0.15 nm was obtained, in favourable agreement with conductance measurements and electrooptical experiments of lipid vesicles. A linear dependency of the activation energy for prepore formation on the applied field is seen, with quantitative agreement between experiment and simulation. The distribution of preparation times suggests a four state pore formation model.

1 Introduction

Membrane electroporation (MEP) is nowadays an established technique to render cell membranes porous and permeable by applying electric pulses to cells in suspension, adherent cells, and tissue. Historically, the structural concept of MEP has been derived from functional changes such as cell death, the nondestructive electro-release of intracellular components from isolated organelles, and the functional direct electro-uptake of naked gene DNA into mouse lyoma cells. MEP is widely used for the efficient direct electro-transfer of all kinds of bioactive substances, in particular drugs and genes, not only in cell biology and biotechnology but also in the new medical disciplines of electrochemotherapy and electrogenetherapy used e.g. for vaccination or in RNA transfection. Other electroporative phenomena such as electrofusion of cells or electroinsertion of xenoproteins by nonpermeabilizing electroporation pulses at low voltages are intrinsically coupled to the structural changes induced by MEP.

Despite major experimental and theoretical efforts, several questions remained unanswered so far: for example, an intermediate on the way to hydrophilic pores has been proposed and tentatively been assigned a hydrophobic pore on the basis of kinetic measurements. Also, conductance measurements on planar lipid bilayers showed the existence of a nonconductive prepore state. However, in molecular dynamics (MD) simulations such a hydrophobic pore has not been observed. Accordingly, the structural features of such an intermediate are still unknown. A second issue is that no attempt has been made so far to quantitatively relate the pore formation times observed in simulations to measured pore formation kinetics, such that up to now the simulations have not been rigorously validated against experiment.

2 Methods

Bilayer patches composed of 128 to 512 POPC (DOPC) lipids surrounded by explicit water were studied. Larger systems were required for the study of equilibrium effects and of a stable electropore. Force field parameters for the lipids were taken from Berger¹ or from Siu et al.². All simulations were performed at full hydration, for 5–200 ns. Ions were considered for the study of membrane capacitance changes under external electric fields as well as for studying a stable electropore.

3 Influence of Electric Fields on Membrane Capacitance

The influence of external electric fields on the membrane capacitance was studied applying external electric fields of 0.1–0.4 V/nm across a DOPC bilayer. In experiments, a quadratic increase of membrane capacitance with the membrane potential was described:

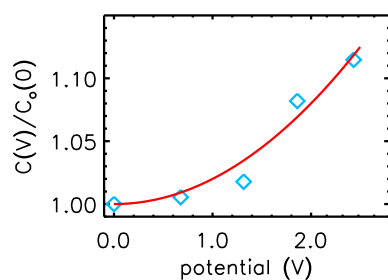


Figure 1. Membrane capacitance changes as a function of the potential drop across a lipid bilayer. The solid line displays the experimentally obtained quadratic behavior, the blue diamonds results from MD simulations applying external electric fields of varying strengths across a DOPC bilayer. The bilayer contained 256 lipids, the phospholipid force field was derived from GAFF³.

$$\frac{C(V)}{C(0)} = 1 + \alpha V^2. \quad (1)$$

Membrane capacitance changes obtained using the recently developed generalized all-atom force field based on Amber (GAFF³) for DOPC are in very good agreement with the experiments. We suggest this modified membrane state with an increased area per lipid and differentially tilted lipid head groups for both lipid leaflets as an intermediate state to prepore formation.

4 Influence of Gramicidin on Membrane Stability

Large potential drops across biological membranes occur already by small charge imbalances. Our results obtained in simulation studies of a DMPC/gramicidin system suggest that gramicidin considerably suppresses membrane electropore formation⁴. Pore formation times were increased at least by a factor of three.

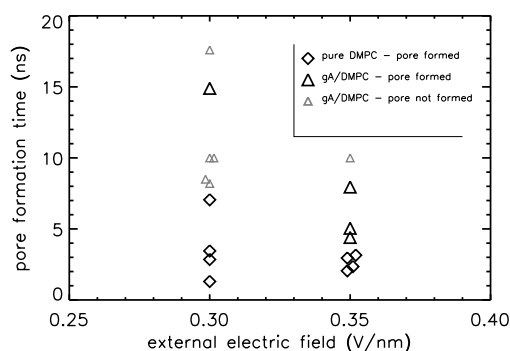


Figure 2. Pore formation times observed in MD simulations for a pure DMPC lipid bilayer (black diamonds) and for a mixed gramicidin/DMPC system (black triangles, gramicidin in DH conformation) at two different field strengths (0.3 and 0.35 V/nm). Small gray symbols denote the simulation lengths of further simulations in which pore formation could not be observed. The total simulation time exceeds 120 ns.

5 Kinetics of Electropore Formation

Comparison of prepore formation rates obtained from 50 simulations at varying field strengths with experiment allowed to estimate the average number of lipids involved in electropore formation to 140. From this result one would predict that pores are separated typically by approximately 7 nm. The radius of stable electropores was estimated to 0.47 ± 0.15 nm from a simulation with properly adjusted external electric field⁵. Pore formation is preceded by two intermediate steps: (1) tilting of lipid headgroups, coupled to an increase in the lipid area; (2) prepore intermediate involving 2–5 lipids⁵.

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